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# Description of a New Species of *Limnonectes* from Sarawak, Malaysian Borneo (Dicroglossidae, Anura)

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**Abstract: A Southeast Asian dicroglossid frog, long known as *Limnonectes laticeps*, has recently been synonymized with *L. khasianus*. The Bornean population of this species is very divergent acoustically from some conspecific populations from the continent. Furthermore, in the mtDNA phylogeny, the Bornean population is nested in a clade with Bornean populations of the *L. kuhlii* complex, and not with the continental *L. khasianus*, which is close to *L. tweediei* and *L. macrognathus*. Because the Bornean population is also divergent morphologically from a continental population and the syntypes of *Rana laticeps*, we describe it as a new species.**

Key words: New species, MtDNA phylogeny, *Limnonectes laticeps*, Sarawak, Taxonomy

## Introduction

*Limnonectes laticeps* was initially described as a member of *Rana* by Boulenger (1882) based on specimens from "Khassya" (=Khasi Hills, Assam). Recently, Ohler and Deuti (2013) relegated the species to a junior subjective synonym of *L. khasianus* (Anderson, 1871), type specimens of which were collected at the same time as the types of *L. laticeps*. The species (as *L. laticeps*) was known only from India at the time of the original description, but was subsequently recorded from many localities of Southeast Asia (Smith, 1925; Bourret, 1942; Taylor, 1962; Inger, 1966; Berry, 1975), and is now considered to widely occur from Myanmar and peninsular Thailand, through Malay Peninsula and Sumatra to Borneo, with the Indian populations isolated to Meghalaya (Khasi and Garo Hills) and Assam (Kaziranga National Park).

Little is known about intraspecific variation in *L. khasianus*. Inger (1966) briefly compared the Bornean population with the type series and noted some morphological differences between them. However, no one has conducted further study since then. Meanwhile, in our field survey in Thailand, Peninsular Malaysia, and Malaysian Borneo, we noticed a large difference in the calls between continental and Bornean populations. In the subsequent survey to clarify their taxonomic relationships through acoustic and molecular analyses, we could not access topotypic Indian samples of *L. khasianus*. Among the localities we surveyed, Thailand is closest to India; therefore we used samples from Thailand as representing continental *L. khasianus* to compare geographic acoustic and genetic variations of continental and Bornean popu-

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lations. We found great divergences between them in both characteristics. We also compared the morphology of syntypes of *Rana laticeps* stored at the Natural History Museum, London (BMNH) with the Bornean specimens and found significant differences between them. We therefore consider the population from Borneo to warrant distinct specific recognition, and describe it as a new species.

## Materials and Methods

DNA sequence data were obtained from tissues frozen or preserved in 99% ethanol (Table 1). Methods for DNA extraction, and amplification and sequencing of the mtDNA fragments are the same as those reported by Matsui et al. (2010). The resultant sequences were deposited in GenBank (Accession numbers AB971130–971139: Table 1). We reconstructed phylogenetic (maximum likelihood [ML] and Bayesian inference [BI]) trees from 2421 base pairs (bp) of partial sequences of mitochondrial 12S and 16S rRNA genes.

We recorded frog calls in the field using a stereo cassette recorder (Sony TC-D5M) with a microphone (Sony ECM-23F), or a digital recorder (Olympus LS-11) at 44.1 kHz/16 bits as uncompressed wave files. The recordings on cassette tapes were digitized at 44.1 kHz/16 bits. We analyzed recordings with SoundEdit Pro (MacroMind-Paracomp, Inc.) and Raven Lite 1.0 for Mac OS X (http://www.birds.cornell.edu/raven) on a Macintosh computer. Temporal data were obtained from the oscillogram and frequency information was obtained from the audiospectrograms using Fast Fourier Transformation (1024 point Hanning window).

For specimens stored in 70% ethanol, we took body measurements mainly following Matsui (1984, 1994): (1) snout-vent length (SVL); (2) head length (HL), from tip of snout to hind border of angle of jaw (not measured parallel to the median line); (3) snout length (SL); (4) eye length (EL), including eyelid; (5) eye-ball diameter (ED), diameter of the exposed portion of the eyeball; (6) head width (HW); (7) internarial distance (IND); (8) interorbital distance (IOD); (9) upper eyelid width (UEW); (10) lower arm and hand length (LAL) from elbow to tip of third finger; (11) forelimb length (FLL); (12) inner palmar tubercle length (IPTL); (13) first finger length (1FL), from distal end of inner palmar tubercle to tip of first finger; (14) third finger disk diameter (3FDW); (15) hindlimb length (HLL); (16) thigh length (THIGH), from vent to tip of knee; (17) tibia length (TL); (18) foot length (FL); (19) inner metatarsal tubercle

Table 1. Sample of *L. khasianus* and other species used for DNA analysis in this study together with the information on voucher, collection locality and GenBank accession numbers. Voucher abbreviations: BORN=BORNEENSIS Collection, University Malaysia Sabah, KUHE=Graduate School of Human and Environmental Studies, Kyoto University; UI=University of Indonesia.

| | | | | |
|---|---|---|---|---|
| 1 | *L. khasianus* | Matang, Sarawak, Borneo, Malaysia | KUHE 10654 | AB971130 |
| 2 | *L. khasianus* | Matang, Sarawak, Borneo, Malaysia | KUHE 10659 | AB971131 |
| 3 | *L. khasianus* | Matang, Sarawak, Borneo, Malaysia | KUHE 42623 | AB971132 |
| 4 | *L. khasianus* | Matang, Sarawak, Borneo, Malaysia | KUHE 53800 | AB971133 |
| 5 | *L. khasianus* | Bala, Narathiwat, Thailand | KUHE 23145 | AB971134 |
| 6 | *L. khasianus* | Bala, Narathiwat, Thailand | KUHE 23158 | AB971135 |
| 7 | *L. khasianus* | Bala, Narathiwat, Thailand | KUHE 23167 | AB971136 |
| 8 | *L.* "*kuhlii*" | Matang, Sarawak, Borneo, Malaysia | KUHE 12025 | AB526322 |
| 9 | *L.* "*kuhlii*" | Kinabalu, Sabah, Borneo, Malaysia | BORN 22645 | AB526323 |
| 10 | *L. tweediei* | Johor, Malaysia | KUHE 52184 | AB971137 |
| 11 | *L. macrognathus* | Ranong, Thailand | KUHE 23923 | AB971138 |
| 12 | *L. kuhlii* | Java, Indonesia | KUHE 26127 | AB971139 |
| 13 | *Fejervarya iskandari* | East Java, indonesia | UI unnumbered | AB526324 |

length (IMTL); (20) first toe length (1TOEL), from distal end of inner metatarsal tubercle to tip of first toe; and (21) fourth toe disk diameter (4TDW). In the univariate comparisons, SVL was compared by Tukey-Kramer test, while the percentage ratios (R) of the remaining characters to SVL were compared by Dunn's multiple comparisons test or Mann-Whitney U test. Because there were no sexual differences in all the characters examined, the sexes were combined and subjected to statistical analyses. The system of description of toe-webbing states followed that used by Savage (1997).

Syntypic specimens of *Rana laticeps* examined for morphological comparisons are stored at BMNH, and other specimens at the Sarawak Research Collections (SRC) and Graduate School of Human and Environmental Studies, Kyoto University (KUHE).

## Results

In the phylogenetic trees obtained, the specimens of *L. khasianus* from Borneo examined here proved to form a clade with the Bornean *L. kuhlii* complex, while those from the continent tended to form a group together with *L. tweediei* (Smith, 1935) and *L. macrognathus* (Boulenger, 1917) (Fig. 1). The Bornean population substantially differed genetically from two lineages of the Bornean *L. kuhlii* complex in the sister clade by large genetic distances (uncorrected p-distance in 16S rRNA of 8.7–10.0%, Table 2), and was even more highly differentiated from the continental *L. khasianus* with p-distances as high as 16.0–16.8%. These values are much higher than those usually observed among good species in this genus (e.g., <8.7% between *L. hascheanus* [Stoliczka, 1870] and *L. limborgi* [Sclater, 1892] [Inger and Stuart, 2010]; 7.9–8.0% between *L. namiyei* [Stejneger, 1901] and *L. fujianensis* Ye and Fei, 1994 [Matsui et al., 2010]).

Bornean and continental populations also greatly differed in acoustic characteristics (see below). Furthermore, the Bornean speci-

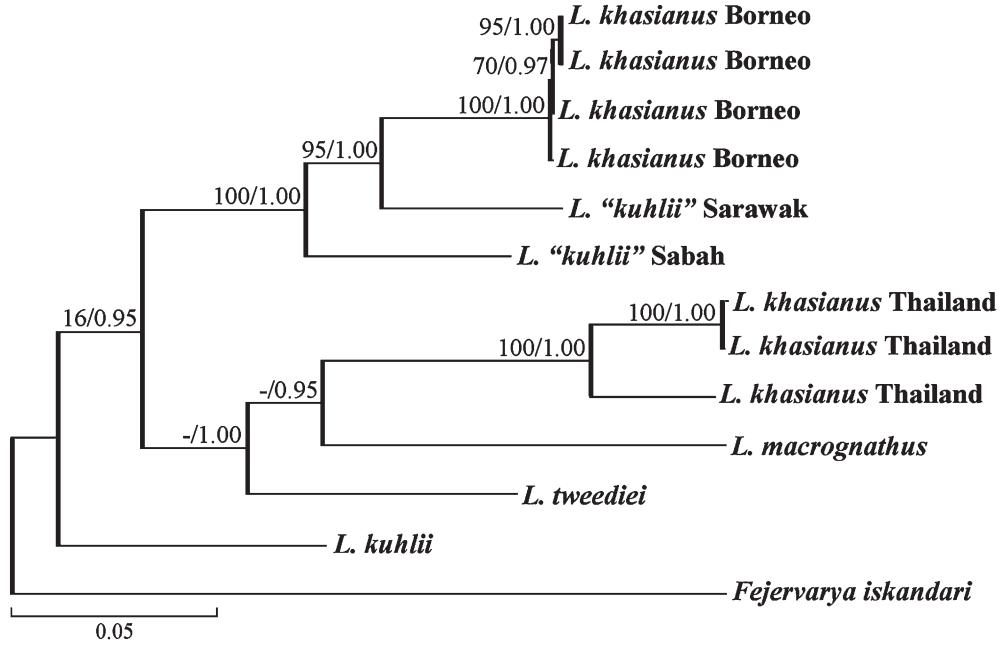


Fig. 1. ML tree from a 2421 bp sequence of mitochondrial 12S rRNA and 16S rRNA genes for samples of *Limnonectes* frogs from Southeast Asia. Numbers above or below branches represent bootstrap supports for ML inference and Bayesian posterior probability (ML-BS/BPP).

Table 2. Uncorrected p-distances (in %) for fragment of 16S rRNA among samples of *L. khasianus* and other species compared.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *L. khasianus* Borneo | | | | | | | | | | | | |
| 2 | *L. khasianus* Borneo | 0.0% | | | | | | | | | | | |
| 3 | *L. khasianus* Borneo | 0.2% | 0.2% | | | | | | | | | | |
| 4 | *L. khasianus* Borneo | 0.3% | 0.3% | 0.1% | | | | | | | | | |
| 5 | *L. khasianus* Thailand | 16.2% | 16.2% | 16.0% | 16.0% | | | | | | | | |
| 6 | *L. khasianus* Thailand | 16.3% | 16.3% | 16.1% | 16.2% | 0.2% | | | | | | | |
| 7 | *L. khasianus* Thailand | 16.8% | 16.8% | 16.6% | 16.6% | 6.4% | 6.5% | | | | | | |
| 8 | *L.* "*kuhlii*" Sarawak | 9.1% | 9.1% | 8.7% | 8.9% | 17.2% | 17.3% | 17.9% | | | | | |
| 9 | *L.* "*kuhlii*" Sabah | 10.0% | 10.0% | 9.9% | 10.1% | 16.9% | 17.0% | 16.8% | 9.8% | | | | |
| 10 | *L. tweediei* | 14.9% | 14.9% | 14.8% | 14.9% | 15.1% | 15.1% | 14.7% | 16.1% | 14.9% | | | |
| 11 | *L. macrognathus* | 17.5% | 17.5% | 17.3% | 17.4% | 15.9% | 16.0% | 17.1% | 17.8% | 17.0% | 15.8% | | |
| 12 | *L. kuhlii* | 14.5% | 14.5% | 14.3% | 14.5% | 16.3% | 16.3% | 16.2% | 15.8% | 14.7% | 15.4% | 15.0% | |
| 13 | *Fejervarya iskandari* | 22.0% | 22.0% | 21.9% | 21.8% | 22.5% | 22.4% | 22.4% | 21.8% | 21.5% | 20.9% | 22.4% | 21.6% |

mens are separated morphologically from the syntypes of *R. laticeps* and the continental ones in congruence with genetic separation. Thus, we conclude that the specimen of *L. khasianus* from Borneo is a distinct species and describe it as follows:

## Systematics

### *Limnonectes hikidai* n. sp.

Figs. 2, 3

*Rana laticeps* Smith, 1925, Sarawak Mus. Jour., 3, p. 32.

*Limnonectes* (*Limnonectes*) *laticeps* Dubois, 1987, Alytes, 5, p. 63. (part).

*Etymology*

The species name is dedicated to Dr. Tsutomu Hikida, Professor of Kyoto University, who is the pioneer of our herpetological survey in Sarawak.

*Holotype*

KUHE 17212, an adult male from Gunung

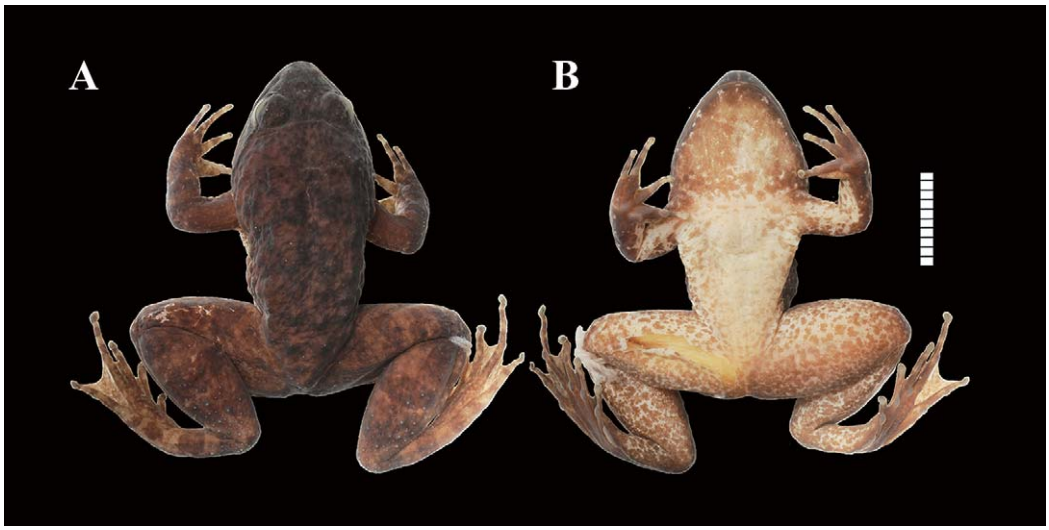


Fig. 2. Dorsal (A) and ventral (B) views of male holotype of *Limnonectes hikidai* (KUHE 17212) after preservation. Scale bar=10 mm.

(=Mt.) Serapi, Kubah National Park, Matang, Kuching District, Sarawak, Malaysia (01°36'25"N, 110°11'27"E, 282 m asl), collected on 13 August 1993 by M. Matsui.

*Paratypes*

A total of 39 specimens all from Gunung Serapi. KUHE 10549, 10550, 4 January 1990; KUHE 10654–10659, 21–22 January1990; KUHE 17001, 22 July 1993; KUHE 17004, 17010, 17011, alt. 300 m asl, 24 July 1993; KUHE 17101, 17105, alt. 300 m asl, 3 August 1993; KUHE 17213, data same as the holotype; KUHE 17224, 17225, 17230, 12 August 1993; KUHE 17248, 15 August 1993; KUHE 17282, alt. 671 m asl, 16 August 1993; KUHE 17302, alt. 640 m asl, 16 August 1993; KUHE 17672, 17673, alt. 397 m asl, 9 September 1993; KUHE 42623, 26 November 2008; KUHE 45789, 45791, 45794, 45796, August 1987 by H. Ota; KUHE 53009, 53010, 13 August 2009; KUHE 53021, 14 August 2009; KUHE 53022-53024, 53027, 53030, 53031, 14 August 2009; KUHE 53160, 26 August 2009; KUHE 53800, alt. 322 m asl, 27 August 2010.

*Referred specimens*

Gunung Mulu: KUHE 10385, 16–20 December 1989. Lanjak Entimau: KUHE 10632, 11 January 1990; KUHE 17452, 28 August 1993. Ranchan, Serian: KUHE 53226, 29 August 2009. Gunung Penrissen: KUHE 48509–48514, 19 December 2013; KUHE 48566–48570, 48586–48588, SRC unnumbered (former KUHE 48589), 21 December 2013; KUHE 48635, 22 December 2013; KUHE 55643, 12 March 2013. Gunung Serapi: KUHE 10551, 4 January 1990; KUHE 12490, 12491, 7 February 1991; KUHE 17067, 3 August 1993; KUHE 17300, 17301, 18 August 1993; KUHE 48728, 26 December 2013; KUHE 48843, 7 March 2014; KUHE 53008, 53013, 13 August 2009; KUHE 54536, below 300 m asl, 28 February 2012; KUHE 55414, alt. 315 m asl, 9 March 2013.

*Diagnosis*

A small species of *Limnonectes* (SVL 31–37 mm in males and 34–40 mm in females); tympanum indistinct; hindlimb relatively short, tibiotarsal articulation of adpressed limb reaching at most to a point between eye and nostril; tips of digits dilated, those on toes partially with horizontal groove; toe webs poorly developed, usually three phalanges free of web on fourth toe; flaps on outer edge of fifth toe and along both edges of second and third fingers not movable. Morphologically similar to *L. khasianus*, but differing from it by having less developed webbing on fourth toe, more rugose dorsum with wrinkles radiating from warts, sharply pointed tusk in males, and usually invisible tympanum, which is largely concealed under skin (vs. dorsum smooth scattered with large tubercles, tip of tusk blunt, and tympanum sometimes visible in *L. khasianus*).

*Description of holotype (measurements in mm)*

Snout-vent length (SVL) 33.0; habitus moderately stocky (Fig. 2A, B); head greatly enlarged, slightly longer (14.2, 43.2%SVL) than broad (14.0, 42.4% SVL); snout obtusely pointed, obtuse in profile, projecting far beyond lower jaw; eye length (4.8, 14.5%SVL) equaling snout length (4.8, 14.5%SVL); canthus rounded; lore sloping, concave; nostril dorsolateral, on canthus, midway between


FIG. 3. Ventral view of left hand (A) and foot (B) of male holotype of *Limnonectes hikidai* (KUHE 17212) after preservation. Scale bar=5 mm.

Table 3. Measurements in adults of *Limnonectes hikidai* sp. nov. and *L. khasianus*. SVL (Mean±1SD, in mm) and medians of ratios (R) of other characters to SVL, followed by ranges in parenthesis. See text for character abbreviations.

| | *L. hikidai* sp. nov. | | *L. khasianus* | | | |
|---|---|---|---|---|---|---|
| | | | India (Type) | | Thailand | |
| | 15 males | 17 females | 4 males | 1 female | 9 males | 4 females |
| SVL | 34.3±1.52 | 36.7±1.94 | 37.4±6.0 | 34.7 | 33.8±4.3 | 32.3±1.8 |
| | (30.9–36.8) | (33.8–40.4) | (33.0–46.4) | — | (28.7–41.9) | (29.7–33.7) |
| RHL | 42.7 | 40.6 | 40.6 | 39.5 | 45.8 | 44.3 |
| | (40.4–44.2) | (39.1–43.1) | (39.6–41.6) | — | (42.9–48.9) | (43.5–45.1) |
| RHW | 41.7 | 39.0 | 41.7 | 41.2 | 45.1 | 43.0 |
| | (38.0–44.1) | (36.9–42.8) | (40.0–46.3) | — | (42.9–47.8) | (42.0–44.4) |
| RIND | 9.7 | 9.6 | 9.9 | 10.7 | 9.9 | 10.0 |
| | (8.9–10.7) | (8.5–10.7) | 9.5–10.3 | — | (8.9–10.5) | (9.2–10.8) |
| RIOD | 9.4 | 9.1 | 9.9 | 8.6 | 9.8 | 8.5 |
| | (9.0–10.6) | (7.2–10.3) | (8.9–10.8) | — | (9.0–10.8) | (8.1–8.8) |
| RUEW | 7.6 | 7.4 | 9.3 | 11.0 | 8.0 | 7.9 |
| | (6.3–8.4) | (6.3–8.8) | (8.9–9.5) | — | (7.6–9.0) | (7.7–8.3) |
| RSL | 14.5 | 14.8 | 18.4 | 19.3 | 16.7 | 16.3 |
| | (13.9–15.7) | (13.4–16.2) | (17.9–19.1) | — | (14.2–18.1) | (15.1–17.3) |
| REL | 14.4 | 14.2 | 12.0 | 15.0 | 15.3 | 15.7 |
| | (12.8–15.2) | (11.8–15.9) | (11.1–13.5) | — | (14.5–15.7) | (14.8–17.2) |
| RED | 11.7 | 11.5 | 13.4 | 15.9 | 12.2 | 12.2 |
| | (10.6–12.6) | (10.5–13.1) | (12.1–14.8) | — | (10.8–18.3) | (11.3–14.1) |
| RTD | 0 | 0 | 7.1 | 6.1 | 6.3 | 6.2 |
| | (0–7.8) | (0–7.0) | (6.4–7.4) | — | (5.5–8.0) | (0–7.1) |
| RT-EL | 0 | 0 | 5.6 | 4.3 | 5.1 | 4.1 |
| | (0–6.9) | (0–3.5) | (5.4–7.1) | — | (4.2–7.6) | (0–4.4) |
| RLAL | 43.9 | 45.4 | 46.2 | 47.0 | 45.5 | 46.4 |
| | (41.4–47.4) | (41.0–48.1) | (44.5–46.7) | — | (45.0–47.9) | (45.4–47.1) |
| RFLL | 56.4 | 58.2 | 59.8 | 61.1 | 59.4 | 57.6 |
| | (52.7–61.9) | (52.5–62.8) | (58.2–62.5) | — | (54.7–61.9) | (55.5–60.1) |
| RIPTL | 4.5 | 4.8 | — | — | 5.3 | 5.2 |
| | (4.0–6.6) | (3.7–6.4) | — | — | (4.8–6.5) | (5.0–5.6) |
| R1FL | 14.2 | 14.6 | — | — | 13.9 | 14.3 |
| | (12.5–15.2) | (13.6–15.6) | — | — | (13.1–15.2) | (13.6–14.8) |
| RTL | 51.4 | 52.1 | 54.6 | 55.6 | 55.6 | 53.9 |
| | (48.6–55.9) | (48.6–54.6) | (50.2–55.3) | — | (51.6–56.4) | (50.4–55.6) |
| RFL | 51.1 | 51.4 | 52.3 | 55.6 | 52.8 | 54.0 |
| | (47.6–54.2) | (47.5–56.0) | (47.0–55.2) | — | (51.3–55.4) | (52.2–55.2) |
| RHLL | 167.8 | 167.3 | 173.0 | 181.6 | 173.1 | 169.0 |
| | (157.7–179.8) | (158.5–179.0) | (161.4–174.8) | — | (160.9–180.2) | (164.4–175.8) |
| RIMTL | 7.3 | 7.2 | 7.2 | 6.9 | 8.0 | 7.7 |
| | (6.2–9.4) | (6.7–8.2) | (6.6–7.4) | — | (7.1–8.9) | (6.2–9.0) |
| R1TOEL | 12.5 | 12.6 | 12.3 | 12.1 | 12.8 | 12.3 |
| | (10.6–13.9) | (11.4–14.8) | (11.8–12.5) | — | (11.6–13.4) | (11.9–12.5) |
| R3FD | 2.0 | 1.9 | — | — | 2.5 | 2.4 |
| | (1.4–2.2) | (1.4–2.2) | — | — | (2.1–3.1) | (2.4–2.7) |
| R4TD | 2.7 | 2.8 | — | — | 3.0 | 2.8 |
| | (2.4–3.0) | (1.7–3.4) | — | — | (2.6–3.5) | (2.7–3.1) |

FIG. 4. Dorsolateral view of a male paratype of *Limnonectes hikidai* (KUHE 17230) in life.

snout and eye; internarial distance (3.2, 9.7%SVL) slightly wider than interorbital distance (3.0, 9.1%SVL), latter slightly wider than upper eyelid (2.7, 8.2%SVL); pineal spot visible; tympanic annulus slightly visible through skin; vomerine teeth in closely set, oblique groups, behind line connecting rear rims of choanae, groups separated from one another by length of one group and from choana by length of one group, lower jaw with a pair of toothlike pointed projections near symphysis, more than twice the depth of mandible at base of projections; tongue oval, deeply notched posteriorly, without papillae; vocal sac and vocal slits absent.

Forelimb thick, relatively short (19.1, 57.9%SVL); fingers moderately slender; finger length formula: II<I<IV<III (Fig. 3A), first finger slightly longer than second; length of first, measured from distal edge of inner palmar tubercle (4.8, 14.5%SVL) equal to length of eye; tips of fingers slightly swollen, forming small pads without circummarginal grooves; no webs between fingers; inner palmar tubercle moderate (1.4, 4.2%SVL), oval, not elevated; middle palmar tubercle oval, smaller than inner palmar tubercle, not contacting inner palmar tubercle; outer palmar tubercle slightly smaller than middle tubercle; proximal subarticular tubercles oval and elevated; distal subarticular tubercles low, flat and indistinct; no supernumerary metacarpal tubercles; edges of second and third fingers with narrow ridges of skin, not freely movable.

Hindlimb thick, moderately long (56.5, 171.2%SVL) about three times length of forelimb; tibia short (17.8, 53.9%SVL), heels not overlapping when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching to anterior corner of eye; foot (17.1, 51.8%SVL) slightly longer than tibia; toe length formula I<II<V<III<IV; tips of toes swollen into distinct, partially grooved small disks (disk diameter of fourth toe 0.9, 2.7%SVL); webbing formula: I 0–1 II 0–1 III 0–3 IV 3–1 V (Fig. 3B); a flap of skin along

outer edge of fifth toe not freely movable; subarticular tubercles oval and distinct; an elongate inner metatarsal, length (2.6, 7.9%SVL), more than half length of first toe (4.6, 13.9%SVL); no outer metatarsal tubercle.

Dorsum rugose, with wrinkles running in all directions, radiating from low, conical warts; warts and wrinkles weak on eyelid and top of snout; weak transverse fold between posterior margins of eyes; strong temporal fold from eye to above axilla; weak dorsolateral ridge from posterior corner of eye to sacral region; warts anterior to anus with translucent spinules; side of trunk with scattered larger tubercles; dorsal surface of hindlimb scattered with small, low warts, tipped with translucent spinules on tibia and tarsus; tarsus with a thick dermal ridge extending proximally from metatarsal tubercle; throat, chest, and abdomen smooth; skin of gular region not modified, scattered with translucent spinules; distinct brownish tinge, but without asperities, forming a nuptial pad covering medial surface of first finger from its base to level of subarticular tubercle.

*Color*

In preservative, dorsum brown irregularly marked with dark brown (Fig. 2A); head with a dark interorbital bar; an oblique blackish brown temporal stripe on and along supratympanic fold from behind eye to above arm insertion; side of head from posterior half of lore to inguinal area pale brown; upper lip light brown with dark brown bars; lower lip dark brown with white spots; limbs marked dorsally with dark-brown crossbars; throat heavily mottled with dark brown (Fig. 2B); chest to abdomen cream spotted with dark brown; ventral side of limbs mottled with brown, especially heavily on posterior thigh and tibia; ventral surfaces of hand and foot dark brown. In life, ventral sides of posterior abdomen and hindlimb lightly covered with yellow.

*Variation*

Individuals of the type series are generally similar to each other in morphology (Fig. 4). Table 3 shows individual variation in size and body proportions. Adult males are not significantly different from adult females in all characters examined including SVL (mean= 34.3±1.5 mm vs. 36.7±1.9 mm; Tukey-Kramer test, $P>0.05$). The point to which the tibiotarsal articulation of the adpressed limb reached varied from the center of the eye (15.8% of males and 11.8% of females) through anterior point of eye (31.6% and 41.2%, respectively) to between eye and nostril (52.6% and 47.1%, respectively). Tympanum is usually not discernible (60.0%), but annulus can be seen in some (33.3%) and clearly visible in a few (6.7%). Degree of toe webbing slightly varies and phalanges free of broad web are 3 (53.3%), 2¾ (40.0%), and 2½ (6.7%) on inner side, and 3 (66.7%), 2¾ (26.7%), and 2⅔ (6.7%) on outer side of fourth toe. A cream-colored mid-dorsal stripe is present in a few specimens (1.9% in Matang and 12.5% in Penrissen). Nearly all specimens have dusty chin marking and dark mottling on ventral surface of hindlimb, and only a few have weak spots or dots.

*Call characteristics*

The advertisement calls of a paratype (KUHE 53160) from the type locality were recorded at the air temperature of 24.1 C. The calls are mainly a single note of 87–148 (mean±SD=108.4±15.4, n=13) ms in duration, but frequently followed by the shorter sub-note of 60–81 (72.3±7.4, n=6) ms (Fig. 5A, B). Calls were intermittently emitted with a long gap (between the first and second main notes) of 3.0–7.4 (4.0±1.2, n=12) s. The call interval, excluding the sub-notes, was 2.9–7.3 (3.9±1.2, n=12) s, and the call (=main note) frequency was about 0.25/s. Calls (the first main notes) consisted of many fine pulses that formed 5–7 (6.1±0.7, n=12) indistinct pulse groups (Fig. 5C, D). The frequency bands were recognized from 0.68–0.92 (0.79±0.06, n=12) to 4.80–5.15 (4.93±0.09, n=12) kHz. Three indistinct groups of harmonic bands were seen in this range, and further indistinct bands were also present up to 6.7 kHz (Fig.

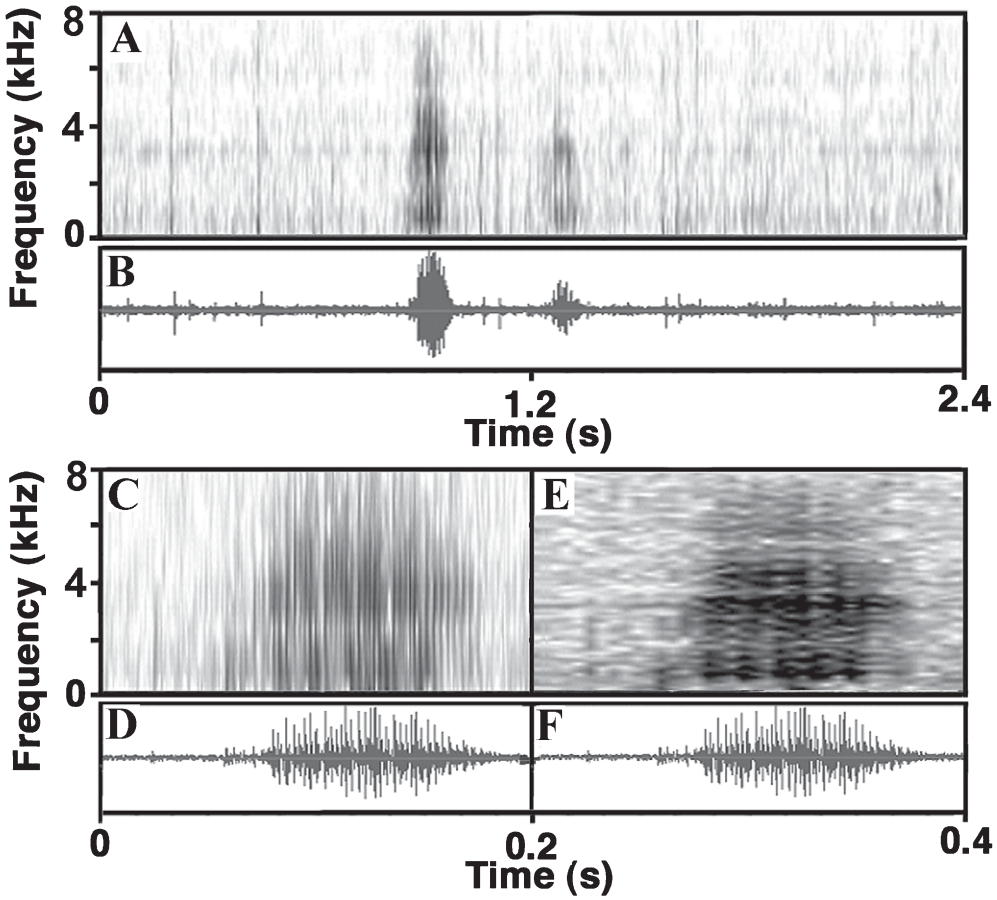


FIG. 5. Spectrograms (A, C, and E) and wave forms (B, D, and F) of advertisement calls of a paratype (KUHE 53160) of *Limnonectes hikidai* sp. nov., recorded at an air temperature of 24.1C. (A, B) A call, showing main and sub-notes, (C-F) a main note showing temporal (C, 128 points) and frequency (E, 512 points) characteristics.

5E, F). The dominant frequency was difficult to define but ranged from 2.98–4.10 (3.42± 0.25, n=12) kHz. The second sub-notes were emitted following the main notes with a gap of 385–495 (430.5±37.7, n=6) ms. They also consisted of many fine pulses that formed 4–5 (4.7±0.5, n=6) indistinct pulse groups. Harmonic bands of higher frequencies were often lacking, but the dominant frequency was similar to that of the main notes, ranging from 3.34–3.51 (3.42±0.06, n=6) kHz.

The call characteristics of a male (KUHE 48566) from Gunung Penrissen were essentially similar to those described above. The calls, emitted with a gap of 1.8–5.4 (3.4±1.0, n=20) s, are mainly a single note of 67–131 (102.5±14.4, n=21) ms in duration, but frequently followed by the shorter sub-note of 58–87 (68.5± 8.6, n=11) ms with a gap of 519–762 (611.6±68.7, n=11) ms, and sometimes by the third note. In the main notes, the frequency bands were recognized from 0.58–0.71 (0.66±0.04, n=21) to 4.15–4.64 (4.40±0.15, n=21) kHz, and dominant frequency was difficult to be defined but the lowest frequency seemed to be fundamental. The second sub-notes were similar to the main notes in frequencies, with bands ranging from 0.62–0.71 (0.67±0.03, n=11) to 3.93–4.60 (4.34±0.19, n=11) kHz.

*Comparisons*

The new species *L. hikidai* is superficially similar to the continental *L. khasianus* with which it was long synonymized, but is differentiated from it by having less developed webbing on the fourth toe (usually three phalanges free of web vs. two phalanges free in the types of *L. khasianus*), more rugose dorsum, sharply pointed tusks in males, and rarely visible tympanum, which is largely concealed under skin (vs. dorsum smooth with scattered large tubercles, tip of tusk blunt, and tympanum sometimes visible in *L. khasianus*). The species is also different in some morphometric characters (Table 3). Results of the statistical test (Dunn's multiple comparison test) indicated that *L. hikidai* sp. nov. had smaller values than the type series of *R. laticeps* in RUEW, RSL, RED, RFLL, and R1TOEL. Also, the new species had smaller values in RHL, RHW, RSL, RED, RLAL, RTL, RFL, and RIMTL than the Thailand population of *L. khasianus*. In addition, the Bornean population was significantly smaller in RIPTL, R3FD, and R4TD than the Thailand population (Mann-Whitney U test, 2-tailed, $P<0.02$). In contrast, the Thailand *L. khasianus* differed from the type series of *L. khasianus* only by larger values of RHL, REL, and RIMTL. Thus, the new species is differentiated from *L. khasianus* by a proportionately narrower head and upper eyelid, shorter head, snout, eye, forelimb, tibia, foot, first toe, and inner metatarsal tubercle than *L. khasianus*, partly confirming a previous report (Inger, 1966 as *L. laticeps*).

More conspicuously, *L. hikidai* sp. nov. is completely different from the continental *L. khasianus* in acoustic characteristics. The advertisement calls of *L. khasianus* from Bala Hala, southern Thailand, recorded at the air temperature of 23.2C, were very similar to the call reported as a rising gurgle by Dring (1979) for a Malay Peninsula population (as *L. laticeps*). The loud call was emitted sporadically and included various types. The typical call consisted of a series of about 16 non-pulsed notes and lasted about 1.7 s. Each note lasted 70–80 ms with the note interval of 40–70 ms; note repetition rate was 8.8. The call had a clear frequency modulation; in the beginning notes, the dominant frequency was about 0.5 kHz, but rose to 1.1 kHz, with harmonics at 2.1 kHz, in the peak note, and finished at 0.95 kHz in the last note. The call also had a marked intensity modulation. Thus, in the temporal characteristics (short call with a single, or sometimes two, notes in the new species vs. long call with many notes in the continental *L. khasianus*), the new species cannot be confused with the continental congener.

The usual lack of visible tympanum of the new species easily differentiates it from all the other congeners except for species of the *L. kuhlii* complex and their close relatives. From members of the Bornean *L. kuhlii* complex, the new species differs in body size and extent of toe webbing (females 34–40 mm in SVL, fourth toe not broadly webbed to disk, flaps on outer edge of the fifth toe and along both edges of the second and third fingers not movable, though usually with a fixed ridge of skin along the fifth toe in the new species vs. females 51–67 mm, usually all of the toes broadly webbed to disks, movable flaps of skin present on the corresponding positions of toes and fingers in the Bornean *L. kuhlii* complex: Inger, 1966). These differences also hold for *L. kuhlii* (Tschudi, 1838) and the other species previously assigned to it (*L. bannaensis* Ye, Fei, and Jiang, 2007; *L. fujianensis* Ye and Fei, 1994; *L. isanensis* McLeod, Kelly, and Barley, 2012; *L. jarujini* Matsui, Panha, Khonsue, and Kuraishi, 2010; *L. sisikdagu* McLeod, Horner, Husted, Barley, and Iskandar, 2011; *L. megastomias* McLeod, 2008; *L. taylori* Matsui, Panha, Khonsue, and Kuraishi, 2010), and their close relatives *L. namiyei* (Stejneger, 1901), *L. asperatus* (Inger, Boeadi, and Taufik, 1996), and *L. fragilis* (Liu and Hu, 1973). *Limnonectes rhacodus* (Inger, Boeadi, and Taufik, 1996) has a tympanum partially obscured by skin, but is smaller (females 21–24 mm) and has the back with numerous transverse wrinkles (Inger et al., 1996).

*Range*

Other than the type locality, Matang, *L. hikidai* sp. nov. has been collected from Gunung Mulu, Miri Division, Gunung Penrissen, Kuching Division, Ranchan, Serian, Samarahan Division, and Lanjak-Entimau Wildlife Sanctuary, Sri Aman Division in Sarawak, Malaysian Borneo. Moreover, the new species has been recorded from Gunung Gadin, Kuching Division (Smith, 1925); Mengiong River, Kapit Division; and Sungei Pesu, Bintulu Division (Inger, 1966) in Sarawak, and Kalimantan of Indonesian Borneo (Veith et al., 2004), and Brunei Darussalam (Das, 2007).

*Natural History*

Some ecological notes have been provided by Inger (1966): the new species is found only in virgin or partly logged rain forest, mostly in aquatic situations, such as in a small stream, consisting of pools (up to ca. 30 cm in diameter and to 4 cm deep, with a silty bottom, strewn with dead leaves and gravel) between rocks and having only a trickle of water except immediately after rains, and in a clayey seepage area at the head of a small stream with leaf litter of about 2 cm deep at the bottom, or in an isolated pool at the top of a hill.

Inger (1966) reported females collected between September and November being gravid. We heard males calling in mid August and mid December at the type locality, Gunung Serapi, and in late December on the slope of Gunung Penrissen. Thus, the breeding season is thought to include August to December. On Gunung Serapi, isolated males were calling at the head of small streams (width<1 m) half submerged in the shallow water (depth<1 cm). On Gunung Penrissen, males were found calling in a roadside concrete ditch, and a pair was found in a slit of the ditch, from where a small amount of water was flowing. Inger et al. (1996) reported the clutch size of the new species to vary from 20 to 45, and size of the largest ova from 2.4 to 2.8 mm. Eggs are black in the animal hemisphere. Eggs laid in nature and larvae are unknown.

Other species found in association with the holotype were: *Leptobrachium* cf. *montanum* Fischer, 1885, *Ingerophrynus divergens* (Peters, 1871), *Ansonia leptopus* (Günther, 1872), *L.* cf. *kuhlii* (Tschudi, 1838), and *L. palavanensis* (Boulenger, 1894).

## Discussion

Although previously reported only from Sarawak outside the continent (Inger and Tan, 1996), frogs identified as *L. khasianus* (as *L. laticeps*) have been recorded from Kalimantan of Indonesian Borneo (Veith et al., 2004), Brunei Darussalam (Das, 2007; Grafe and Keller, 2009), and Sumatra (Amir Hamidy, personal communication). From their geographic proximity, the populations from Kalimantan and Brunei likely represent *L. hikidai* sp. nov. By contrast, we lack information on *L. khasianus* from Sumatra, which is very close to the Malay Peninsula, where *L. khasianus* occurs. However, Sumatra has different faunal elements, i.e., its endemic elements, elements common to Java to Bali, and elements occurring widely on the islands nearby and the Malay Peninsula, that must be closely related to historical invasions into the island (Matsui et al., 2014). Thus, we are at present not sure whether or not the Sumatran population is conspecific with *L. hikidai* sp. nov. or *L. khasianus*.

Unfortunately, no genetic or acoustic information of topotypic north Indian populations of *L. khasianus* is available. However, the Thai population is geographically close to them and was tentatively treated as a representative of continental populations in this study. Distribution of *khasianus* in Myanmar, situated between the Indian type locality and Thailand, is not clear, but seems to be limited to the eastern area (the Dawna Hills, Schwe Settaw on the Shan and Mandalay border, and Kachin Hkakabo National Park: IUCN, 2013), far distant from the Indian localities of Meghalaya and Assam, but close to Thailand. Because of this great geographic gap from the type locality and significant morphometric differences detected (see above), populations

of *L. khasianus* from Thailand and Malay Peninsula, as well as Myanmar, may represent a distinct, unnamed species.

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